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GRANT NUMBER DAMD17-96-1-6056

TITLE: Fatigue in Persian Gulf Syndrome-Physiologic Mechanisms

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REPORT DATE: July 1997

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
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DTIC QUALITY INSPECTED 4

REPORT DOCUMENTATION PAGE

OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 1997	Annual (15 Jun 96 - 14 Jun 97)

4. TITLE AND SUBTITLE Fatigue in Persian Gulf Syndrome-Physiologic Mechanisms

5. FUNDING NUMBERS

DAMD17-96-1-6056

6. AUTHOR(S)

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The University of Texas Southwestern
Medical Center at Dallas
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, MD 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

19990610137

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

We hypothesize that the common complaint of abnormal fatigue and exercise intolerance in these patients is attributable to impaired energy production via oxidative phosphorylation. Under this general hypothesis, we will address three specific questions: 1) Is there an abnormality of muscle oxygen utilization or oxygen transport to muscle during exercise in affected individuals? 2) Is there exaggerated metabolic muscle fatigue in exercise consistent with impaired energy production? 3) Is the metabolic and physiologic response to aerobic physical conditioning impaired in these patients.

In order to address these questions, we will employ forearm and cycle exercise to determine maximal work and oxidative capacity and to compare fatigue and metabolic responses to similar relative workloads among patients and age and weight matched sedentary control subjects; and we will compare muscle metabolic and physiologic responses to aerobic training in patients and matched control subjects. We will monitor oxidative metabolism by employing 31-phosphorus magnetic resonance spectroscopy; and by utilizing near infrared spectroscopy. In a cohort of patients and control subjects we will evaluate the hypothesis that oxidative limitations detected with non-invasive testing is attributable to impaired function of the mitochondrial metabolism as assessed biochemical in biopsied muscle.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Gulf War Illness	12
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Ronald G. Hall 7/28/98

PI - Signature Date

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Introduction

A variety of illnesses have been linked to military service in the Persian Gulf Conflict, but no consistent medical syndrome has been recognized and no specific etiology is known. Muscle symptoms, in particular abnormal fatigability and myalgias, are common in affected individuals, but the etiology of these muscle symptoms is unknown. We are investigating the general hypothesis that a fundamental physiologic mechanism of muscle fatigue in Gulf War veterans is an impairment of muscle oxidative metabolism. Under this general hypothesis, we are addressing four specific questions:

1) Is there exaggerated metabolic muscle fatigue in exercise consistent with impaired energy production?

2) Is there an abnormality of muscle oxygen utilization or oxygen transport to muscle during exercise in affected individuals?

3) Is the normal increase in muscle oxygen utilization and the capacity for oxygen transport in response to regular, aerobic physical conditioning impaired in these patients?

4) Is there a specific pattern of impaired activities of mitochondrial enzymes to account form impaired oxidative metabolism or attenuated increases in oxidative capacity in response to physical training?

Our study will enroll 25 Gulf War veterans with prominent symptoms of fatigability and myalgia and matched control subjects employing resources of a center devoted to the study of human muscle metabolic disorders and to investigation of the physiologic basis of chronic fatigue.[1-3]. The study employs protocols and non-invasive monitors of oxygen transport and utilization as well as detailed muscle biochemistry employed in our laboratory to identify specific causes of exercise intolerance in patients referred to our center. These include measurement of systemic oxygen transport (cardiac output) at rest and in exercise by means of acetylene rebreathing;[4]. monitoring of muscle metabolism by 31 phosphorus magnetic resonance spectroscopy;[5-7]. and monitoring of muscle oxygenation by means of near infrared spectroscopy.[8, 9].

Body

Experimental methods, procedures:

Subject identification, recruitment: . We have proposed to identify and recruit 25 affected veterans and 25 control subjects.

Experimental procedures: The fundamental approach to evaluating muscle oxidative metabolism in Gulf War veterans involves cycle and forearm exercise testing during which physiologic and metabolic changes related to muscle oxidative capacity are monitored:

a) *Cycle exercise*. Subjects undergo resting and exercise evaluation of oxidative metabolism using cycle ergometry. Studies are designed to assess peak capacity for oxygen utilization and oxygen transport (cardiac output) as well as monitoring changes in blood levels of metabolites that reflect levels of anaerobic glycogenolysis (blood lactate and lactate/pyruvate ratios) as well as heart rate and blood pressure responses to graded exercise.

b)*Aerobic forearm exercise*. Subjects also undergo aerobic forearm exercise, monitoring the pattern of contractile fatigue and changes in venous effluent metabolites that reflect the rate of glycogenolysis and adenine nucleotide breakdown via adenylate deaminase.

c) *Near infrared spectroscopy (NIRS)* . Direct evaluation of oxygen extraction over working muscle is evaluated utilizing NIRS performed during repetitive hand gripping exercise sampling oxygenation of the flexor digitorum profundus. Light in the NIR range (700-1000 nm) passes readily through biological tissues including skin and bone. NIR light is diffusely scattered by tissues and photons are absorbed primarily by the iron-porphyrin complexes of oxy- and deoxyhemoglobin and -myoglobin and by oxidized copper atoms of cytochrome aa3. NIR is able to detect *qualitative* changes in the reduction-oxidation state of the copper complex of cytochrome aa3 (in cytochrome c oxidase) and oxygenation of tissue hemoglobin (Hgb) and myoglobin (Mgb). Thus, NIR spectroscopy provides a unique opportunity to evaluate noninvasively local muscle O_2 extraction and the state of mitochondrial redox in muscle relative to oxygen extraction from circulating blood and myoglobin. This technique permits detection of muscle oxidative defects[9, 10].

d)*[31] Phosphorus magnetic resonance spectroscopy* (^{31}P MRS). ^{31}P MRS permits measurement of *intracellular* metabolites of relevance to muscle energy metabolism 5 major phosphorus peaks are found in resting muscle: orthophosphate (Pi), phosphocreatine (PCr), and 3 peaks corresponding to the α, β, and γ phosphates of ATP. Peak height and area correlate with the relative concentrations of the respective metabolite.[11, 12] The ß peak typically is used to estimate concentrations of ATP and by convention is assumed to represent 5.5 mM per kgm wet weight of muscle.[13, 14] Phosphorus MRS has identified a number of abnormalities in patients with respiratory chain defects. At rest muscle PCr levels are often low and Pi may be elevated.[15-18] This result has been interpreted to indicate that oxidative

phosphorylation is deficient even at rest. With exercise there is typically an exaggerated fall in PCr and rise in Pi relative to work performed consistent with impaired oxidative phosphorylation. After exercise, recovery of PCr typically is greatly delayed, consistent with the oxidative deficit.

e) *Muscle biopsy - histochemical and biochemical evaluation.* We have proposed to evaluate histologic and biochemical in 10 patients with fatigue and myalgia and 10 controls and to evaluate the change in muscle metabolic capacity in response to training.

f) *Aerobic training* - we have proposed to enroll 10 patients and 10 control subjects in a 10 week period of training to assess physiologic and metabolic adaptation and to test the hypothesis that the subjects with symptoms of fatigability show altered capacity to adapt to conditioning exercise.

Results and Discussion

The original annual report for this project submitted in July, 1997 was faulted on the basis of slow progress and because little data was contained in that report. A revised report was requested. This request virtually coincided with the submission of the grant report for the current year. Therefore, with respect to many of the questions raised for the report originally submitted 7/97, reference is made to information contained in the current report submitted for 7/98.

Subject identification, recruitment: . The critique faults the progress of the proposal with respect to the pace of subject recruitment. As indicated in the original report, our approach to subject recruitment was modified from our original proposal to permit the identification of veterans followed at the Dallas VA Medical Center with neuromuscular symptoms. This change was undertaken for two major reasons: 1) First, we determined that identifying local veterans would vastly improve subject availability for testing, and in particular would facilitate the conduct of the training portion of our protocol. 2) Secondly, by establishing a collaboration with Dr. Haley, we would be able to utilize epidemiological surveys that would employ factor analysis of symptoms and a history of toxin exposure during service in the Persian Gulf. Such an epidemiological approach, we reasoned, would enhance the likelihood of identifying a specific pattern of symptoms of fatigability, myalgias, and weakness in Gulf War veterans, a consistent pathophysiology of these symptoms, and potential causal links between exposure to specific risk factors and symptoms.

The initial survey identified 107 veterans including 18 veterans with predominantly neuromuscular symptoms and 18 asymptomatic control subjects. From this initial survey we were able to recruit 13 symptomatic and 11 asymptomatic subjects for our study. Subsequently we have surveyed an additional 200 subjects from whom we are recruiting the remaining subjects to complete our study (see annual report, 7/98). This approach to subject recruitment has necessitated a request for a 18 month no cost extension of the grant. However, we believe that the additional information that will be obtained from the study will greatly enhance its value.

Time commitment of the PI and Co-PI. The reviewer suggests that the slow pace of data acquisition relates to a lack of commitment to this project on the part of the investigators. The reviewer refers to previously expressed concerns that the time commitment of the investigators is too low. The major reason for the slow pace of the study in the initial year was the fact the process of identifying local subjects for participation - including administering and analyzing the survey instruments and identifying, contacting, recruiting, and scheduling subjects - took a good deal longer than anticipated. This delay in start-up necessitated our request for an 18 month no-cost extension of the study which has recently been approved. The experiments are now proceeding at a good pace. The disadvantage of a longer time necessary for completion of the study will be offset by the benefits of a more informative and definitive investigation of the basis of neuromuscular symptoms in Gulf War veterans. The investigators are, in fact, fully committed to this project and are enthusiastic about the preliminary data.

Reviewer critique in relation to specific hypotheses:

1. Is there exaggerated metabolic muscle fatigue in exercise consistent with impaired energy production? The reviewer indicates that inadequate information was provided to assess progress on this question.

Our protocol has utilized hand grip and cycle exercise to address this question and have obtained data on 24 subjects. For handgrip exercise, we have collected data on initial grip force and change in grip force during fatiguing aerobic exercise. We also have collected data on changes in venous lactate and ammonia with such exercise. During peak cycle exercise we have monitored oxygen uptake, cardiac output, and have calculated systemic a-v O2 difference. Additionally during cycle exercise, we have monitored changes in blood lactate, pyruvate, the L/P ratio, and potassium. Analysis of these data indicates that there is a significantly lower capacity for large muscle (cycle) exercise in symptomatic compared to asymptomatic veterans whereas tolerance of hand grip exercise was similar in both subject groups (see annual report dated 7/98).

2. Is there an abnormality of muscle oxygen utilization or oxygen transport to muscle during exercise in affected individuals? The reviewer states that this question does not seem to have been addressed since no data were presented.

We have addressed this question in 24 subjects studied initially and in 4 subjects studied after completion of aerobic training by utilizing cycle ergometry and measuring oxygen utilization and cardiac output (systemic O_2 transport) during peak exercise and by calculating peak systemic arteriovenous O_2 difference. Mean values for oxygen utilization, cardiac output, and systemic a-v O_2 difference were all lower in patients compared to controls, but none of these differences reach statistical significance (see annual report date 7/98). We also have evaluated the integrity of oxygen delivery in relation to oxygen utilization during forearm exercise using near infrared spectroscopy. No qualitative differences in NIR spectroscopy have been identified in the 24 subjects studied to date.

3. Is there a normal increase in muscle oxygen utilization and the capacity for oxygen transport in response to regular, aerobic physical conditioning in these patients? The reviewer pointed out that this question had not been addressed in the original report.

We have since enrolled 7 veterans (5 patients, 2 controls) in the training portion of our study, and 4 of these (2 patients, 2 controls) have completed 10 weeks of cycle training. All of the subjects who have completed the aerobic conditioning phase of this study have demonstrated an increased oxidative capacity attributable to increased cardiac output or increased peak systemic a-v O_2 difference (see annual report dated 7/98).

4. Is there a specific pattern of impaired activities of mitochondrial enzymes to account form impaired oxidative metabolism or attenuated increases in oxidative capacity in response to physical training? The reviewer states that inadequate information was required to assess progress on this question.

We have now performed needle muscle biopsies on 24 subjects during the initial evaluation. In addition we have performed needle biopsies at the end of the aerobic training protocol on 4 subjects.

Conclusions

Modification of the procedure of subject identification and recruitment from the original protocol has increased the time required to complete this study. However, we conclude that a factor analysis of symptoms and epidemiological survey of potential toxin exposure enhances the significance of results from this study and provides a highly objective methodology for identifying veterans experiencing symptoms of fatigue, myalgia, and weakness from veterans without neuromuscular or other symptoms. Adoption of the needle biopsy technique for acquisition of biochemical and morphologic data and expanding the number of subjects on whom such data will be collected will enhance the capability of detecting and determining the significance of possible differences between symptomatic and asymptomatic veterans.

Evaluation of data to date indicates that Gulf War patients with symptoms of abnormal fatigability have a statistically significant reduction in cycle work capacity and a trend toward a lower peak capacity for oxygen utilization, cardiac output, and a-v O2 difference which do not reach statistical significance. While there is also a trend to lower work capacity in aerobic hand grip exercise in patients, differences do not attain statistical significance.

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